

Morphological Approach to Hair Disorders

Rodney Sinclair,¹ Damian Jolley,² Rica Mallari,¹ Jill Magee,³ Antonella Tosti,⁴ Bianca Maria Piracinni,⁴ C Vincenzi,⁴ Rudolf Happle,⁵ Juan Ferrando,⁶ Ramon Grimalt,⁶ Leroy Thérèse,⁷ Dominique Van Neste,⁷ Abraham Zlotogorski,⁸ Angela M. Christiano⁹ and David Whiting¹⁰

¹Department of Dermatology, University of Melbourne, Australia; ²School of Health Sciences, Deakin University, Melbourne, Australia; ³Mayne Health Dorovitch Pathology, Melbourne, Australia; ⁴Department of Dermatology, University of Bologna, Italy; ⁵Department of Dermatology, University of Marburg, Germany; ⁶Hospital Clinic, University of Barcelona, Spain; ⁷Skinterface, Tournai, Belgium; ⁸Department of Dermatology, University of Marburg, Germany Hadassah Medical Center, Jerusalem, Israel; ⁹Department of Dermatology and Genetics and Development, Columbia University, New York, USA; ¹⁰Baylor Hair Research and Treatment Center, Dallas, Texas, USA

The Workshop on the morphological approach to hair disorders brought together a group of clinicians involved in hair biology research. Six speakers spoke on a range of topics that can be grouped broadly into a central theme. It summarizes the evolution of medical research. The section by Tosti and coworkers describes a patient with a new unique syndrome. The section by Ferrando and colleagues provides a framework in which patients with rare hair disorders can be classified. The section by Whiting tries to define the normal anatomy of the hair follicle and both horizontal and vertical sections. It is only when normal anatomy has been absolutely defined that pathological deviations can be recognized. The section by Sinclair and coworkers attempts to estimate the reliability of histological diagnosis

so that its true value of pathology can be recognized. The section by Zlotogorski and coworkers shows how accurate clinical and histological definition of disease acts as the cornerstone for gene discovery techniques. Once a causative mutation is found and a gene product identified, then the biological consequences of the altered protein product can be studied and the impact of the abnormal molecular function on hair biology can be understood. It is hoped that improved understanding of hair disease will then lead to useful therapeutic interventions. The final section by Leroy and Van Neste highlights the difficulties of evaluating therapeutic interventions in hair loss disease and proposes a new technique. Keywords: Blaschko/papular atrichia/scalp biopsy histology. JID Symposium Proceedings 8:56–64, 2003

This sequence of papers tells a story. It is that medical research begins and ends with the patient. In all disciplines of medicine patients present to doctors who by careful observation of the signs and symptoms of their complaint establish a diagnosis. The needs of our patients motivates us to go into the laboratory and research their disease and find useful therapeutic interventions that we can then take back to the patient to alleviate morbidity.

In our discipline, interaction between clinicians who are morphologists and diagnosticians and scientific researchers is pivotal in workshops such as this one help to increase the understanding between these two groups and foster closer collaboration.

WHAT'S NEW ON HAIR DISORDERS: ALOPECIA DUE TO SCALP MOSAICISM FOLLOWING BLASCHKO'S LINES (TOSTI, A)

Tosti *et al* presented the case of a woman with a unique hair loss disorder. She presented with two areas of hypotrichosis on the scalp which had been present since childhood and were fixed. At

the age of 1 the child had been noted to gradually develop linear hyperpigmentation on the head, neck, trunk and limbs. The hair on the left parietal region and on the occipital area stopped growing at this stage and remained short in contrast to the adjacent scalp hair. These patches had hair 3–5 cm in length that was pigmented and had a lanugo like texture (**Fig 1**).

Scalp and skin biopsies were performed. The skin biopsy showed mild epidermal acanthosis with follicular plugging (**Fig 2**). Scalp biopsies were sectioned longitudinally and horizontally. Longitudinal sections showed the presence of terminal follicles. Sebaceous glands were hyperplastic. Transverse sections showed a decreased follicular density with numerous vellus and intermediate follicles.

A diagnosis of scalp mosaicism associated with widespread cutaneous mosaicism due to systematized sebaceous nevus was made. Treatment with 2% topical minoxidil twice a day produced gradual elongation and thickening of the affected hairs with considerable cosmetic improvement according to the patient.

Alopecia due to scalp mosaicism is a feature of incontinentia pigmenti, where the areas of alopecia follow Blaschko's lines. Unlike this case, the involved areas are completely bald. In this case the scalp mosaicism produced a population of hair that differed from surrounding scalp because of the length and texture. The alopecic areas in fact represented short lanugo-like pigmented hair. The pathology confirmed the presence of terminal and intermediate follicles with mild reduction in hair density. The histological findings were consistent with a diagnosis of nevus

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Rodney Sinclair, University of Melbourne, Department of Dermatology, St. Vincent's Hospital, 41 Victoria Parade, Fitzroy 3065, Melbourne, Victoria, Australia. Email: sinclair@svhm.org.au.

Abbreviations: AGA, androgenetic alopecia; APL, Atrichia with papular lesions; CE-PTG, contrast enhanced phototrichogram technique.



Figure 1. Hypotrichosis on the left parietal region with short pigmented hairs.

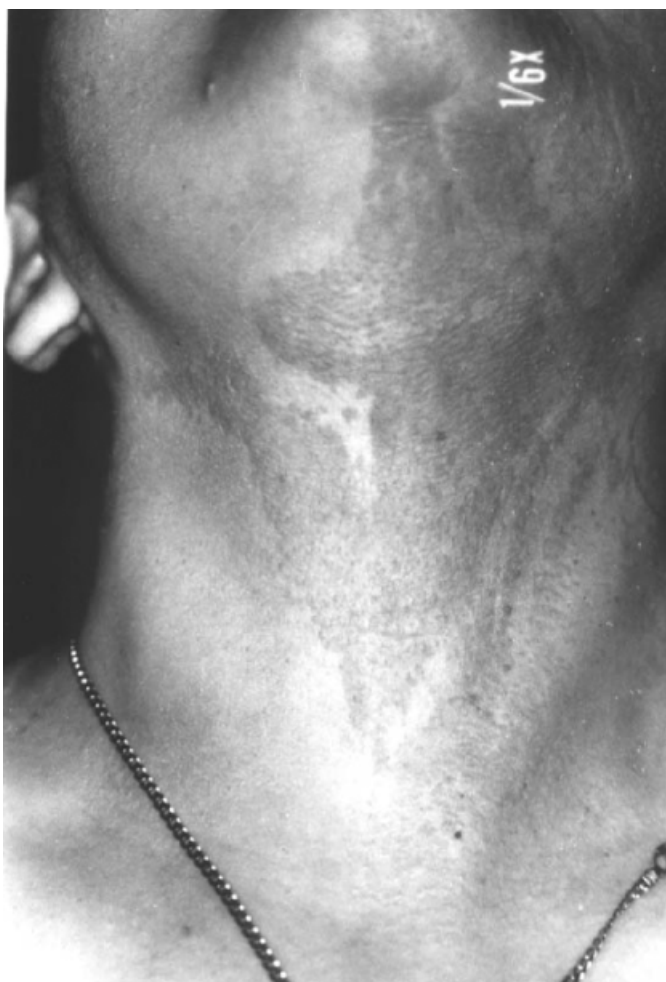


Figure 2. Yellow-brown hyperpigmentation following the Blaschko's lines.

sebaceous because of the hyperplasia of the epidermis, the increased number of large and piriform sebaceous glands and the presence of ectopic apocrine glands in the mid dermis. The authors were unable to find any comparable cases in the literature.

This case points out that the presence of bands or patches of scalp hair of a different texture and length with respect to the surrounding scalp may indicate scalp mosaicism.

AN APPROACH TO THE DIAGNOSIS OF HAIR DYSPLASIAS (FERRANDO, J)

Hair diseases represent a significant portion of cases seen by paediatric dermatologists. Many disorders of the hair can be studied with simple diagnostic techniques, as the hair is easily accessible to examination. While numerous techniques for examination of the hair are now available and include scanning electron microscopy, X-ray microanalysis of genetic hair disorders, in general, clinical observation and a microscopic examination of the hair shaft provide the greatest clues. Whilst X-ray microanalysis and chromatography of hair amino acids are useful in the diagnosis of trichothiodystrophy, this diagnosis can often also be made by polarized light microscopy. With light microscopy specific abnormalities are easily detected with careful observation. The range of abnormalities seen include periodic narrowing of hair (monilethrix), ringed hair (pili annulati), trichoschisis and "tiger tail" hair (trichothiodystrophy), pili torti, trichorrhexis invaginata (Netherton syndrome), bubble hair and "ruffling" (loose anagen hair).

Whilst uncombable hair syndrome may be most easily diagnosed with scanning electron microscopy, clues are also obtained with light microscopic examination of hair shafts, in particular when they are examined in cross-section.

Hair dysplasias in children can be oriented by clinical observation and microscopic examination of hair shafts. Numerous atlases are available to facilitate this diagnosis. (Ferrando and Grimalt, 2000).

THE MORPHOLOGY OF THE HUMAN HAIR FOLLICLE IN VERTICAL AND HORIZONTAL SECTIONS (WHITING, D)

Hair growth Established human scalp hair follicles cycle independently and continuously during their lifespan through stages of growth, rest and shedding (Montagna and Parakkal, 1974; Abell, 1994; Stenn *et al*, 1999; Stenn and Paus, 2001). Scalp hairs comprise large terminal hairs and small vellus hairs. Terminal hairs are conspicuous and exceed 0.03 mm in diameter and 1 cm in length, and may be pigmented and medullated. The hair fiber diameter remains uniform during a single growth phase under normal conditions. Terminal hairs grow to a specific length, which varies with the individual. Hair length is determined by the rate and duration of the growth phase (Whiting, 2001). Vellus hairs are inconspicuous and are 0.03 mm or less in diameter and less than 1 cm in length and lack melanin and medulla. Terminal hairs miniaturized to vellus hair proportions are termed vellus-like hairs. Terminal hairs are rooted in subcutaneous tissue or deep dermis. Vellus hairs are rooted in upper dermis. Termination of the growing or anagen phase is marked by the intermediate or catagen phase which lasts approximately 2 weeks. In catagen, the hair shaft retreats upward, and the outer root sheath shrinks. In catagen, the lower follicle disappears leaving an angiofibrotic strand or streamer (stela) indicating the former position of the anagen root. The ensuing telogen phase lasts an average of 3 months before a new anagen hair develops. In telogen, the resting club-shaped, depigmented root is situated at the bulge level where the arrector pili muscle inserts into the hair follicle. The telogen hair is shed during the exogen phase, which may not coincide with the new anagen phase. Assuming 100,000 scalp hairs with 10% in telogen, the average hair loss equals 100 per day. The next anagen cycle begins with enlargement of the dermal papilla at bulge level and formation of a new anagen bulb.

Human hair follicle anatomy

Terminal anagen hair The terminal anagen hair extends from its bulb in the subcutaneous tissue to its point of emergence from the epidermis through the follicular infundibulum (Montagna Solomon, 1994, and Parakkal, 1974; Headington, 1984; Sperling, 1991; Abell, 1994; Solomon, 1994; Whiting, 2000).

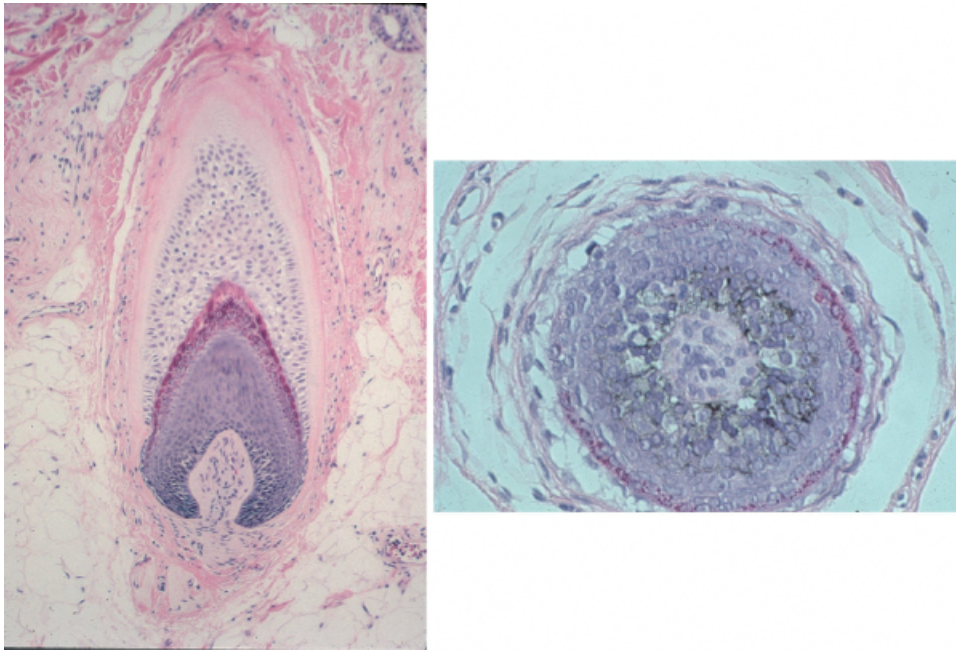


Figure 3. Hair bulb: The dermal papilla is surrounded by successive layers of hair matrix cells with melanocytes, inner root sheath with unkeratinized Huxley's layer and the outer keratinizing layer of Henle, external root sheath (trichilemma), hyaline membrane, and fibrous sheath.

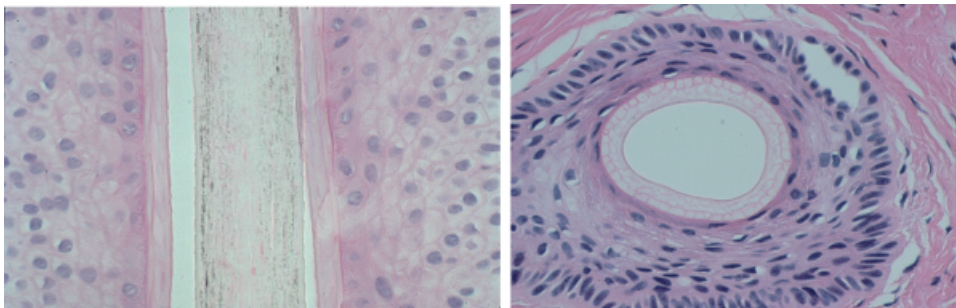


Figure 4. Upper half of lower follicle: Hair shaft is surrounded by fully keratinized, inner root sheath encircled by outer root sheath (trichilemma).

Hair bulb The root consists of the hair bulb, which surrounds the dermal papilla containing connective tissue cells and blood vessels (**Fig 3**). The papilla is surrounded by undifferentiated, actively dividing hair matrix cells. Melanocytes are usually present at the apex of the dermal papilla. Hair matrix cells in this vicinity give rise to hair medullary cells. Hair matrix cells around this central area produce elongated cortical cells which stream upward to form the developing hair shaft. Higher up in the keratogenous zone, these cells become compacted into hard keratin. The outer fringe of matrix cells forms the hair cuticle and the surrounding inner root sheath. The hair cuticle invests the hair fiber with 6–10 overlapping layers of cuticle cells. Cuticle cells keratinize and project outward and forward to interlock with the inwardly projecting cuticle cells of the inner root sheath. The inner root sheath surrounds the hair fiber and comprises 3 layers: The inner layer forms the cuticle of the inner root sheath comprising overlapping elongated cells which slant downward. The middle layer of Huxley comprises 3–4 layers of cuboidal cells. The outer layer of Henle comprises a single layer of elongated cells. The inner root sheath is surrounded by one or more layers of cells of the outer root sheath or trichilemma. The potential space between inner and outer root sheaths is named the companion layer and allows the inner root sheath to slide upward over the outer root sheath during hair growth. The outer root sheath is covered by the hyaline, or vitreous membrane, which is continuous with the epidermal basement

membrane surrounding the dermal papilla. This in turn is surrounded by the connective tissue or fibrous sheath of the hair follicle that is continuous with the dermal papilla. A pad of elastic tissue, the Arao-Perkins body, may develop under the dermal papilla (Arao and Perkins, 1969).

Lower follicle The central hair shaft grows upward through the lower and upper follicle. Proceeding from hair bulb up the lower follicle, the inner and outer root sheaths thicken and become well demarcated. Henle's layer keratinizes first with the appearance of trichohyaline granules near the bulb, forming a distinct pinkish keratinized band higher up from the bulb. The cuticle of the inner root sheath is the next to keratinize, synchronizing with keratinization of the cuticle of the hair shaft. Finally, trichohyaline granules appear in Huxley's layer, signaling impending keratinization. Keratinization of the inner root sheath is completed half way up the lower follicle (**Fig 4**). The keratinized inner root sheath occupies the upper half of the lower follicle. The lower follicle ends at the level of insertion of the arrector pili muscle, the so-called bulge area (Cotsarelis *et al*, 1990).

Isthmus The isthmus extends upward from the bulge area to the level of entry of the sebaceous duct. The inner root sheath crumbles and disappears in the isthmus of the upper follicle (**Fig 5**). There it is replaced by trichilemmal keratin formed by the outer root sheath. Trichilemmal keratin lines the upper

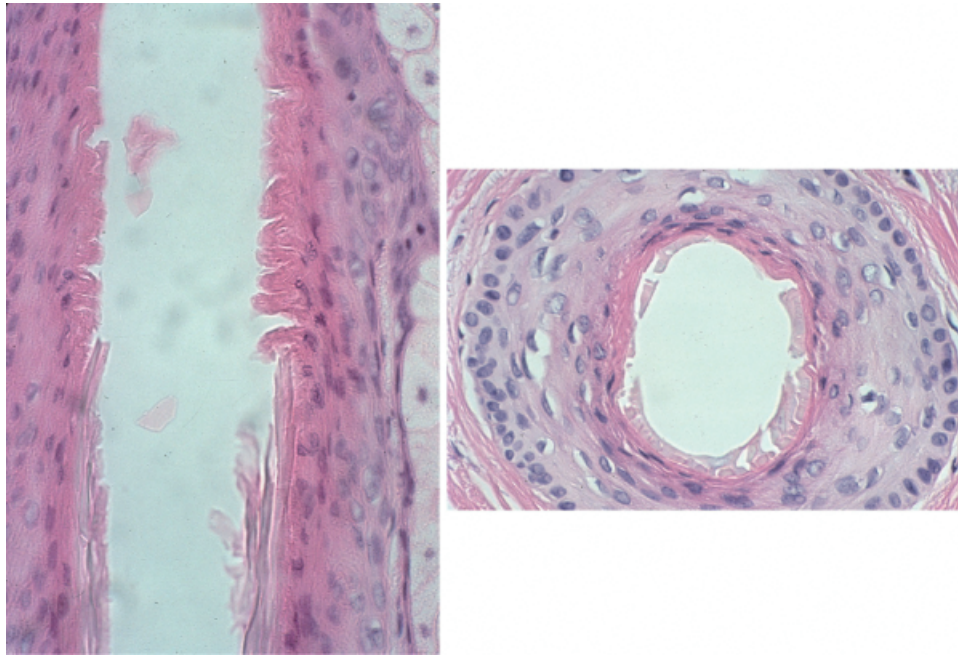


Figure 5. Isthmus: The greyish, inner root sheath fragments and disappears and is replaced by pinkish trichilemmal keratin.

isthmus extending to the entry of the sebaceous duct at the base of the infundibulum.

Infundibulum The infundibulum extends upward from the sebaceous duct level to surface epidermis. The infundibulum is lined by epidermis with a granular layer and basket-weave keratin which is continuous with skin surface epidermis. The hair shaft has no secure attachments to isthmus or infundibulum, which allows freedom of movement.

Follicular units Horizontal sections at the sebaceous duct level show up follicular units. Follicular units are hexagonal tissue packets surrounded by a loose collagen network containing several terminal and vellus follicles with sebaceous ducts and glands and arrector pili muscles.

Vellus hair Vellus hairs are rooted in papillary or upper reticular dermis. Vellus hairs do not contain medullary cavity or melanin. Vellus hair diameter is less than the thickness of its inner root sheath. True vellus hairs have thin external root sheaths and stela in the upper dermis. Vellus-like miniaturized hairs have thicker external root sheaths and long stela extending into lower dermis or fat. Hairs are typically miniaturized by androgenetic alopecia or by alopecia areata. Follicular stela, in upper dermis only, indicate vellus hairs. Follicular stela in lower dermis indicate terminal, catagen, or telogen hairs or miniaturized, vellus-like hairs.

Terminal catagen and telogen hair

Catagen When anagen ends, hair goes into catagen, the intermediate stage between growth and rest, for 10–14 days. As catagen begins, the hair shaft and bulb start retracting upward leaving behind an angiofibrotic streamer or stela linking the follicle to the site of the former anagen bulb. The hair shaft and inner root sheath slide upward together through outer root sheath leaving an elongated mass of trichilemmal outer root sheath below. Apoptosis or individual cell death of trichilemmal cells produces a volumetric shrinkage of the outer root sheath. Thickening and wrinkling of the surrounding hyaline layer occurs with this shrinkage of trichilemma. As the hair shaft retreats further upward, its base becomes club shaped and surrounded

by a pocket of trichilemmal keratin. The vestigial bulb and dermal papilla trail beneath, linked to the follicular stela.

Telogen As the telogen hair develops, it retracts to the level of the bulge at the insertion of the arrector pili muscle (**Fig 6**). Here a telogen germinal unit is formed below the telogen club. The telogen germinal unit consists of trichilemma that is somewhat convoluted and surrounded by palisading basaloid cells. The telogen germinal unit has a characteristic appearance and shows no obvious apoptosis. A central mass of trichilemmal keratin, star-shaped in horizontal section, surrounded by trichilemma and fibrous sheath, is present between the hair shaft and telogen germinal unit. Discrimination between terminal anagen, catagen, and telogen hairs is only possible when the lower follicle is examined below the bulge level for the presence of inner root sheath, apoptosis, or trichilemmal club, respectively. In the upper follicle only a keratinized hair shaft can be seen with no internal root sheath, so discrimination between anagen, catagen, or telogen hairs is not possible at this level. After 2–4 months of telogen, a new anagen hair bud develops beneath the telogen germinal units and grows down the existing follicular tract or stela to form an anagen hair. Subsequent hair cycling will continue throughout life for as long as that hair follicle is viable.

Follicular counts

Accurate counts of hair follicles are often useful in diagnosing different causes of hair loss (Whiting, 1998). Detailed follicular data can be derived from examination of horizontal sections of scalp biopsies. All terminal and vellus hairs, follicular streamers (stela), and follicular units should be counted. Anagen, catagen, and telogen terminal hairs can be distinguished. 4 mm punch biopsies, from the mid-scalp of normal controls, have shown a mean follicular count of 40 hairs comprising 35 terminal hairs and 5 vellus hairs (Whiting, 1998); the terminal hairs comprised 93.5% anagen and 6.5% telogen hairs; the average follicular density was approximately 3 follicles/mm².

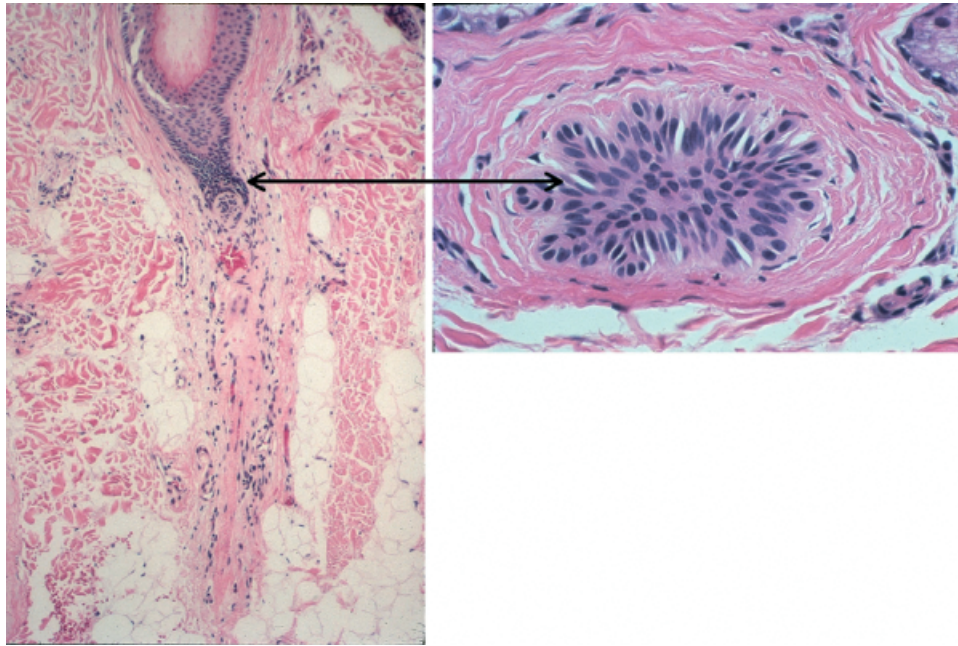


Figure 6. Telogen hair: In vertical section, the telogen club with its sac of trichilemmal keratin has retracted upwards, trailing trichilemma, dermal papilla and follicular streamer (stela) below. The horizontal section shows the irregular island of basaloid cells which represents the telogen germinal unit.

Conclusions

Visualizing the human hair follicle at different levels and in different points of the hair cycle should allow easy correlation between vertical and horizontal sections. Working knowledge of the normal three-dimensional appearance of the human hair follicle should assist in recognizing the various abnormalities that can occur in clinical or experimental situations.

THE RELIABILITY AND REPRODUCIBILITY OF HORIZONTALLY SECTIONED SCALP BIOPSIES IN THE DIAGNOSIS OF CHRONIC DIFFUSE TELOGEN HAIR LOSS IN WOMEN (SINCLAIR, RD)

Women with androgenetic alopecia may present with a diffuse reduction in hair density on the crown, chronic increased telogen hair shedding, or both (Sinclair, 2001). In women the frontal hair-line tends to be preserved, whereas men with androgenetic alopecia usually develop bitemporal recession early. Women who present with chronic increased telogen hair shedding without any discernable reduction in hair density over the crown may have either chronic telogen effluvium or androgenetic alopecia and scalp biopsy may be required to differentiate these two conditions (Whiting, 1996).

Chronic telogen hair loss may occur as a primary idiopathic condition (chronic telogen effluvium) or may be secondary to a range of occult nutritional, endocrine, autoimmune or iatrogenic causes. Routine investigations usually identify secondary causes; however, primary chronic telogen effluvium is a diagnosis of exclusion and a scalp biopsy is required to exclude early androgenetic alopecia (Sinclair, 1999).

The histological hallmark of androgenetic alopecia is miniaturization of terminal follicles into vellus-like follicles (Headington, 1984). Vellus-like follicles have been defined as hairs with a hair shaft diameter ≤ 0.03 mm and thinner than its inner root sheath (Headington, 1984). The shaft lacks pigment and a medullary cavity. Secondary vellus follicles are distinguished from primary vellus follicles by the presence of an erector pili muscle and follicular streamers. As this distinction is difficult on horizontal sections all hairs are counted as vellus-like hairs whether primary or secondary to miniaturization from any cause (Whiting, 1993).

A 4-mm punch biopsy taken from the mid frontal scalp and sectioned horizontally is considered to be the best way to assess follicular miniaturization and detect early androgenetic alopecia (Whiting, 1998). Given that androgenetic alopecia is a patterned disorder that preferentially affects the frontal scalp in women and spares the occipital scalp, biopsy from the frontal scalp is most likely to demonstrate the histological features of androgenetic alopecia in affected women.

Based on studies in men, a reduction in the ratio of terminal to vellus-like hairs from greater than 6:1 to fewer than 4:1 is seen in androgenetic alopecia (Whiting, 1993). Similar changes are seen in women and in a review of 219 horizontally sectioned biopsies from women with androgenetic alopecia, the average ratio of terminal to vellus hairs was 2.2:1 (Whiting, 1998).

To determine the value of horizontally sectioned scalp biopsy in the distinction between androgenetic alopecia and chronic telogen effluvium a number of assumptions need to be tested. These include: that chronic telogen effluvium is a distinct disease and not merely a prodrome to androgenetic alopecia; that pathologists can accurately identify and count vellus hairs in a horizontal scalp biopsy; and that a single 4 mm scalp biopsy is an adequate sample size. To test this last assumption a prospective estimate of the reliability of a single 4 mm scalp biopsy in the diagnosis for androgenetic alopecia in women was undertaken, based on repeated observations from the same woman.

Two hundred and seven women presenting with chronic diffuse hair loss had three 4 mm punch biopsies taken from immediately adjacent skin on the vertex scalp. All 3 biopsies were sectioned horizontally. Findings were compared with 305 women who underwent two biopsies, with one sectioned horizontally and the other vertically. The terminal to vellus-like ratio (T:V) at the mid-isthmus level was used to diagnose androgenetic alopecia (T:V $< 4:1$), chronic telogen effluvium (T:V $> 8:1$) or indeterminate hair loss (T:V = 5:1, 6:1 or 7:1).

Among the 305 women who had a single horizontal scalp biopsy, 181 (59%) were diagnosed with androgenetic alopecia, 54 (18%) with chronic telogen effluvium and 70 (23%) as indeterminate.

Six hundred and twenty-one horizontal biopsies were assessed from 207 patients. On the basis of consensus over 3 biopsies, 159 (77%) were diagnosed with androgenetic alopecia, 44 (21%) as

chronic telogen effluvium and the remaining 4 women (2%) as indeterminate. Using each single biopsy as the criterion for diagnosis, 398 (61%) were classified as androgenetic alopecia, 99 (16%) as chronic telogen effluvium and 124 (20%) as indeterminate. In 493 (79%) biopsies, the single biopsy conclusion was identical to the 3 biopsy conclusion. Where disagreement was seen (21%), most were classified as indeterminate rather than a wrong diagnosis. Of the 477 "true" androgenetic alopecia biopsies, only 3.3% were wrongly classified as chronic telogen effluvium.

Application of these diagnostic criteria achieved accurate diagnostic definition in 98% of women with triple horizontal biopsies vs. 79% with single horizontal biopsy. Clinicians using biopsy to distinguish between androgenetic alopecia and chronic telogen effluvium should be aware of the potential limitations of this procedure and consider doing more than one 4 mm punch biopsy for horizontal section.

MOLECULAR GENETICS OF PAPULAR ATRICHIA (ZLOTOGORSKI, A)

Atrichia with papular lesions Atrichia with papular lesions (APL, papular atrichia, OMIM#209500) is a rare form of irreversible alopecia which is inherited in an autosomal recessive pattern. In individuals affected with this form of hair loss, hairs are typically absent from the scalp, axilla and body, and patients are almost completely devoid of eyebrows and eyelashes. APL patients are unique in that, along with total atrichia, papules and follicular cysts filled with cornified material start to appear during the first years of life, particularly on the scalp, face and extremities.

Genetics Recently, this form of atrichia was linked to chromosome 8p12 (Ahmad *et al*, 1998; Nothen *et al*, 1998; Sprecher *et al*, 1998), and the first four mutations were reported (Ahmad *et al*, 1998; Cichon *et al*, 1998; Zlotogorski *et al*, 1998). During the next four years 11 further frameshift, nonsense and missense mutations (including the two presented here) have been identified in the human *hairless* (*HR*) gene in families around the world (Ahmad *et al*, 1999a, c; Kruse *et al*, 1999; Sprecher *et al*, 1999a; Sprecher *et al*, 1999b; Aita *et al*, 2000a; Zlotogorski *et al*, 2001), establishing the pathogenetic role of *h* in APL. Heterozygous individuals have normal hair, and are clinically indistinguishable from genetically normal individuals.

In a family (mother and son) previously described from Australia (Sinclair & DerBerker, 1997), thought to have an autosomal dominant form of APL, we demonstrated the pseudodominant inheritance of this disease. Both affected members were homozygous for the same mutation, designed as R33X. The parents originated from the same small village, and the deceased father was a potential carrier of this mutation. In another family with APL from Germany, 2 different mutations were found in 2 affected sisters (2847delAG and Q1176X). The parents and 2 healthy daughters each carried one mutation in *HR* and therefore were not affected. These are the first reports of pseudodominant and compound mutations leading to APL.

Histology Histological examination of affected scalp skin shows the absence of mature hair follicle structures, sparsely or normal distributed sebaceous glands and follicular cysts (Kanzler and Rasmussen, 1986). Variations in the structure and shape of hair follicle remnants have been described in patients with APL (Ahmad *et al*, 1999c). There are 2 types of keratinous cysts; large thin-walled and small thick-walled cysts (Nomura *et al*, 2001). The wall of the large cysts usually showed epidermoid keratinization (infundibular origin) with formation of keratohyalin granules. These cysts contained cornified material in their cavities. On the other hand, the small cysts showed the trichilemmal keratinization, suggesting that they originated

from the isthmus. These cysts presumably develop from the malformed or incomplete hair follicles.

Animals Hairless mice develop a normal first pelage up to the age of about 14 days, at which they begin to lose their hair in a frontal to caudal wave. Hair loss is completed in one week, and the hair never regrows. Examination of skin biopsy showed the absence of normal hair follicle structure and dermal cysts. In 1989, the human disease, APL, was first proposed as a homolog of the *hairless* mouse mutation (Sundberg *et al*, 1989). The *hairless* mutation mapped to mouse chromosome 14 (Sundberg, 1994) and was demonstrated to be due to a proviral insertion in intron 6 of the *HR* gene that leads to abnormal splicing. The similarities in phenotype between hairless mice and humans with APL facilitated the discovery of the *hairless* gene on chromosome 8p using comparative genomics (Ahmad *et al*, 1998). Further mutations have been described in the *HR* gene in mice, responsible for the more severe rhino (*hr^{hr}*) and Yurlovo (*hr^{rhY}*) phenotypes (Panteleyev *et al*, 1998).

Function The precise function of hairless protein remains elusive. The hairless gene product encodes a putative single zinc finger transcription factor with restricted expression in the brain and skin of mouse, rat and human, which is directly regulated by thyroid hormone. It is postulated that the absence of hairless protein initiates a premature and aberrant catagen due to abnormal signaling that normally controls catagen-associated hair follicle remodeling. Recently, it was shown that hairless is associated with the nuclear matrix, suggesting a role in transcriptional regulation (Djabali *et al*, 2001).

Summary APL is considered to be a rare disease. However, the prevalence of this disease is probably underestimated, as shown by the increasing number of reported families during recent years. Therefore, it is likely that congenital atrichia with papular lesions is far more common than previously thought and often mistaken for the putative autoimmune form of alopecia universalis. To clarify this discrepancy, a set of criteria for the clinical diagnosis of congenital atrichia with papular lesions has been proposed (Zlotogorski *et al*, 2002). These criteria are based on personal observations of 10 Arab families and the retrospective analysis of other families described in the literature. APL is one of the many forms of congenital alopecia (Sinclair *et al*, 1997). Patients are sometimes born without hair and none ever grows, but more typically, patients are born with normal hair that sheds (from the front to the back of the scalp) after several months (or a few years) and never regrows. APL patients are unique in that, along with total hair and body atrichia, papules and follicular cysts start to appear during the first years of life in a typical distribution on the scalp, face (specifically, around and under the eye) and extremities (around the elbows and knees). The distribution of these papules does not correspond to the haired locations on the body (e.g., under the eye, around the elbow). There is some minor inter- and intrafamilial variation in the age of onset of hair shedding, the amount and distribution of papules and type of cysts. In some patients, hypopigmented whitish streaks are found on the scalp surface. So far, in all patients described in the literature, consanguinity has been documented, thereby establishing the autosomal recessive nature of this disease (Zlotogorski *et al*, 1998). No associated nail, teeth, sweating or thyroid abnormalities have been reported in APL families, helping to distinguish APL from other forms of ectodermal dysplasia that may be associated with atrichia. There are no growth or developmental problems in APL families, except in one family with 2–3 years delay in bone age (Kruse *et al*, 1999). Laboratory tests including biopsy findings of absence of mature hair follicle structures, cysts filled with keratinous material and pathogenetic mutations in the *HR* gene, associated with a typical history of lack of response to any treatment modality, help in establishing the final diagnosis. We assume that increased awareness of APL

and the use of these guidelines will facilitate the recognition of this disease and will help to establish its diagnosis.

It is of interest that in some families with a completely identical clinical picture, associated with autosomal recessive pattern of inheritance, no mutations were found in the *HR* gene. This suggests locus heterogeneity in congenital atrichia with papular lesions, or alternatively that other diseases may share this clinical picture. Further characterization of hairless gene mutations as well as other mutations in related genes in mice and human will facilitate our understanding of the regulation of hair growth and cycling.

CONTRAST ENHANCED PHOTOTRICHOGRAM PINPOINTS SCALP HAIR CHANGES IN ANDROGEN SENSITIVE AREAS OF MALE ANDROGENETIC ALOPECIA (VAN NESTE, D.)

In male androgenetic alopecia (AGA), global changes of scalp hair observed on many years are the result of discrete structural and/or functional modifications occurring at the level of individual hair follicles. Phototrichogram is a noninvasive technique to monitor functional aspects of hair follicles; the method has been first reported in the early 70s and has been subject to various technological improvements (reviewed by Van Neste, 1999). Considerations about its feasibility and usefulness in the hair clinic have highlighted questions about what we can see and what we need to see (Van Neste, 1993). After a series of preliminary observations and reports (Rushton *et al*, 1993; Van Neste and Rushton, 1997), we showed that a contrast enhanced phototrichogram technique (CE-PTG) was able to detect all transitions of thick and thinning follicles from anagen through catagen, telogen phases. Also, if photographed sites were subsequently subject to biopsies, individual hair fibres seen at the scalp surface could be traced down into transversally sectioned scalp samples. Growth stage and hair thickness ($\leq 40 \mu\text{m}$ or $> 40 \mu\text{m}$) were almost perfectly matched on a follicular basis (Van Neste, 2001).

The aims of this study were To confirm in a series of male subjects with AGA, various natural hues of hair color that accurate hair counts on scalp close-up photography should be obtained after CE.

To evaluate the total and growing hair fraction and to characterize the importance of hair miniaturization in relation with the usual clinical staging of patterns (modified Norwood – Hamilton scale).

The salient finding reported in this study of male subjects with AGA is the fact that CE-PTG quantifies specific and early functional disturbances of hair follicles situated in scalp areas prone to balding. Therefore, the CE-PTG method can now be considered as a gold standard for calibration of other tools such as an innovative scalp coverage scoring (SCS) method that is even more user- and patient-friendly (Skinterface patent, 2001).

Study protocol Five controls (18–25 years) and 21 untreated male subjects with AGA (16–51 years) were examined and classified according to a modified Hamilton-Norwood scale [AGA stages I–II ($n=9$); stage III ($n=9$); stage V ($n=3$)].

Each subject was asked to visit the clinic twice at a 48-h interval.

On the first visit, hairs were clipped on 3 predefined anatomical sites of 1 cm^2 area. Two sites were located on the top of the head (left and right side at least 7 cm apart), i.e., in an area prone to develop AGA. One occipital site clinically unaffected in the presently selected AGA patterns was also explored.

At both visits, scalp photographs (primary enlargement $\times 3$) were taken in all subjects before and after application of transient hair dye for CE (Van Neste, 2001).

From baseline photographs, we established total hair density (all visible hair/ cm^2) obtained from two different photographic procedures, i.e., without and with CE.

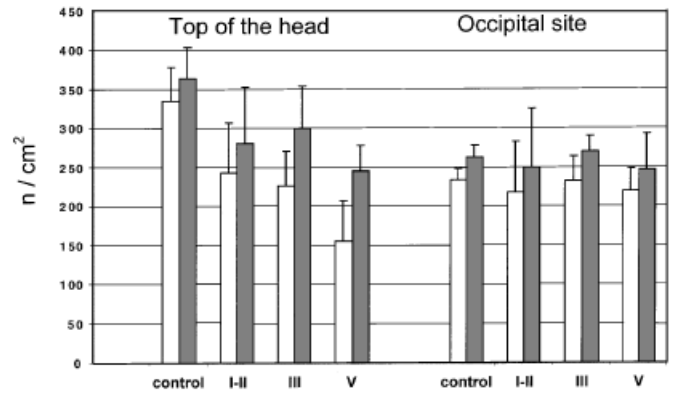


Figure 7. Effect of contrast enhancement on total hair density in control and AGA subjects (groups I–V). Total hair density (all visible hair/ cm^2 ; mean \pm SD) is measured without CE (white bars) and with CE (gray bars) in top of head (left panel) and occipital sites (right panel).

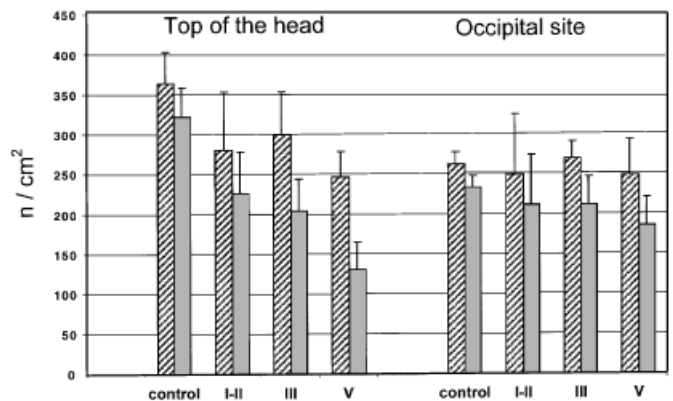


Figure 8. Total and anagen hair counts in control and AGA subjects (groups I–V). Total (hatched bars) and anagen (gray bars) hair density (n hair/ cm^2 ; mean \pm SD) are measured after contrast enhancement in top of head (left panel) and occipital sites (right panel).

From the combination of CE-photographs taken at baseline and two days later we established the anagen hair density (all visible growing hair/ cm^2) to assess hair growth and to generate the phototrichogram data (CE-PTG). We also dot marked thin hair ($= 40 \mu\text{m}$ against a calibrated micrometric ruler) in the top of head site photograph showing the largest increase in hair counts after CE. In the AGA subjects, we then compared the proportion of thin hair detected on CE-PTGs with the thin hair population as measured with transmission light microscopy (subsample of 60 hair clipped at baseline and displayed on glass slides for diameter measurements against a micrometric ruler).

Results For all but three sites ($n=75/78$), we observed more hair after CE (**Fig 7**) with a maximum increase of 84% on the top and 36% on the occipital sites.

On the top of the head, the AGA prone sites showed significant numbers of extra hairs with CE (expressed in percentage) increasing specifically ($p<0.0001$) from controls (8.5%) to AGA stages I–II (14.3%), III (27.8%) and V (47.8%). On occipital sites, however, CE detected also extra hairs but differences were unrelated to clinical categories: controls (11.9%), AGA stages I–II (12.8%), III (15.3%) and V (11.8%). In control subjects, a higher hair density (with or without CE) was noted on top sites versus occipital sites ($p<0.0001$). On top sites, there was a decrease of hair density in the AGA group as compared to controls (control $>$ AGA stages I–V; $p<0.0001$), as opposed to occipital sites, where no significant change was detected.

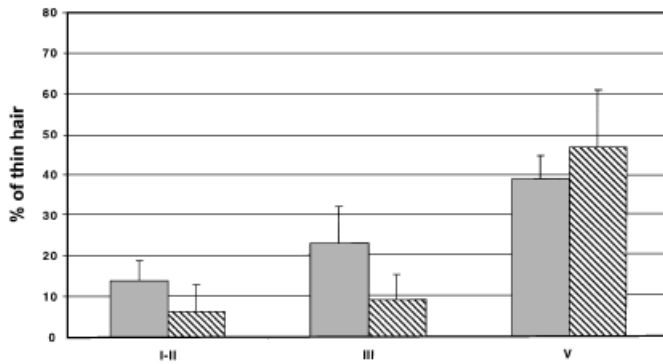


Figure 9. Proportion of thin hair ($\text{Ø} \leq 40 \mu\text{m}$) in top of the head sites of AGA subjects (groups I–V). The percentage of thin hair (mean \pm SD) is measured with two distinct methods: evaluation with a ruler on CE-PTG (gray bars) and microscopy on clipped hair (hatched bars).

The comparative examination of anagen hair density versus total hair counts (Fig 8) detects an even more pronounced decrease of growing hair density in relation to clinical stages in the AGA prone sites (control $> \text{I–II} = \text{III} > \text{V}$; $p < 0.0001$), while subtle but statistically not significant changes were present in the occipital site of the most severely affected AGA group V.

Thin hair counts increased significantly according to AGA staging I–V ($p = 0.0051$), and in the mildest forms (I–III), thin hairs were more effectively detected on CE-PTGs ($p = 0.0057$) as compared with microscopic measures on clipped hair samples (Fig 9). If we analyze separately the hormone sensitive (top of the head) sites (left panels in Figs 7, 8) of the AGA group, we observe a significant increase of thin hair percentage ($p = 0.0002$) and a significant decrease of anagen hair density ($p = 0.014$) and percentage ($p = 0.0004$) in relation with increasing severity. Interestingly, total hair counts with the PTG technique without CE detects gradually less hair in the more severely affected subjects ($p = 0.0307$) as opposed to the CE-PTG ($p = 0.6$).

Discussion It may appear trivial to report that more scalp hair is detected after CE, but the method has only been introduced very recently in the field of scalp hair evaluation (Van Neste *et al*, 1997). Depending on the individuals, light colored thick hair with a normal anagen phase, as well as very thin hair (down to $8 \mu\text{m}$) with short anagen duration is being detected only with the CE-PTG method (Van Neste, 2001).

In this context, as shown in Fig 9, we have to underline that the measurement of percentage thin hair in the clipped hair sample may no longer be considered as reliable due to the less accurate sampling (clipping, collection, displaying...). Hence, any sensitive instrumental method of clipped hair samples may lead to erroneous conclusions because of lack of control of the sampling process itself. We would like to emphasize that this method was unable to detect the effects of a 12-month treatment course of finasteride (double blind controlled study vs. placebo) in man with AGA even though the diameter of 64558 clipped hair fibres had been analyzed (personal unpublished data).

While the thinnest hair is not cosmetically important, it may have diagnostic or prognostic significance. Indeed, in AGA the thin hair population probably constitutes a significant therapeutic target and as such could be predictive of therapeutic responses that may be considered as clinically relevant information.

The use of CE-PTG on fixed scalp sites allows some speculation about the expansion dynamics of AGA. Indeed a previously published follow-up study using various scalp locations (not affected sites at baseline in AGA subjects) demonstrated a progressive reduction of hair counts, probably due to a shortened anagen phase eventually associated with a delayed hair regrowth (Courtois *et al*, 1994). The present study

confirms that AGA subjects have decreased hair counts as compared to controls, but in the androgen sensitive sites only (Fig 7). Also, using the more accurate CE-PTG approach (calibrated against scalp biopsies (Van Neste, 2001)), we document in a range of stages of AGA no further reduction of scalp hair density (Fig 7), but a reduction of anagen duration (Fig 8) and eventually a transformation into thin hair production (Fig 9). Both events are more clearly expressed in the most severely affected AGA group V.

We hypothesize that two processes may be involved: one is shortening of the anagen phase; the other is reduction of the hair diameter. The genetic background will decide whether the two processes occur at the same time or not: in the former the follicle will rapidly enter into atrophy; in the latter a more progressive and chronic process will develop. Depending on the number of follicles in the androgen sensitive area that choose for one or the other option there will be either a dramatic installation of severe balding or alopecia will progress slowly over many years.

This dual process hypothesis may be of importance when the potential of therapeutic response of a single hair follicle is contemplated. Therefore, long-term follow-up studies of individual hair fiber production are required.

We conclude that the CE-PTG allows an early detection of decreased (total and growing) hair density and hair miniaturization in scalp areas prone to balding in male subjects with AGA, confirming its potential use as a gold standard method for calibration of other tools. Such a study using an innovative SCS method (Skinterface patent, 2001) showed that SCS scores in a target site were correlated with CE-PTG data: positively with the percentage of thick anagen hair ($r = 0.86$) and negatively with density of thin hair ($r = -0.68$) in that same site (Van Neste *et al*, 2003).

CONCLUSION

These six studies each reflect different aspects of hair research. It takes us on a journey that begins with careful clinical observation of the morphology of a patient's hair complaint to establish a diagnosis. Patients' needs motivate us to research their disease and find novel therapeutic interventions to take back to the patient to alleviate morbidity. Interaction between clinicians who are hair morphologists and scientific researchers is pivotal. Hair morphology research involves many aspects.

Careful observation of the physical presentation of the patient and correlation of these findings with the histological features of biopsy specimen enhances the specificity of both. Many conditions associated with structural abnormalities of the hair fiber are caused by single gene mutations. Cataloguing these conditions based on their clinical presentation and hair microscopy findings allows them to be methodically studied. Some inherited disorders are amenable to gene discovery procedures. Reverse genetics, whereby the causative mutation is found, the gene product identified and the biological consequences of the altered protein product studied, allows determination of normal and abnormal molecular function and sheds light on the requirement of normal follicle function.

Hair morphology also involves sub-classifying patients who present with multifactorial disease on clinical grounds. Female patterned hair loss is an example of a multifactorial disease whereby careful observation of the patients' clinical presentation helps predict which patients are likely to respond to therapies.

One issue that confounds all clinical hair research is the difficulty of accurately monitoring hair growth, monitoring the hair cycle and documenting patient response to therapy. Improvements in photographic methodology have allowed clinical trials to be conducted into the treatment of androgenetic alopecia, but further work is required, particularly in the assessment of scarring alopecia, alopecia areata and the study of normal hair growth and cycling.

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